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Mudflat soil amendment by sewage sludge: Soil physicochemical properties, perennial ryegrass growth, and metal uptake

INTRODUCTION

Mudflats (also known as tidal flats) are valuable land resources located in the interaction zone between land and sea and are found in many parts of the world (Wang and Wall 2010). The fast pace of cropland loss in China is causing alarm over food security and China's ability to remain self-reliant in crop production (Yu and Lu 2006). Mudflats are important alternative croplands in China. About 1.1–1.2 million ha of mudflats have been reclaimed to croplands in the past 50 years (Cao and Wong 2007). It is estimated that additional 1.0–1.5 million ha of mudflats will be reclaimed to cropland by the 2050s, according to the current sediment deposition rate of river deltas. The newly reclaimed mudflats are low in fertility as indicated by poor soil structure, extremely low organic matter (OM) content, low nutrient level and lack of microbial diversity, which is not suitable for cultivation. The keys to mudflat reclamation to arable lands are (1) to reduce salinity and (2) to increase the soil OM content and thus soil fertility.

The former determines if the reclaimed mudflat can be used for crop production and the latter determines if the crop production is sustainable. Salinity reduction is often accomplished through freshwater irrigation. Fertility enhancement is usually realized through instantaneous application of a great amount of OM because soil natural OM formation is extremely slow.

Sewage sludge is a by-product in the process of centralized wastewater treatment. Safe disposal of sewage sludge is a challenge to the world. At present the main methods of sludge disposal and comprehensive utilization include incineration, landfill, ocean dumping and land application (Fytili and Zabaniotou 2008). Land application of sewage sludge has a great incentive in view of soil amendment and nutrient recycling and reuse including OM, nitrogen (N), phosphorus (P) and other plant nutrients (Logan and Harrison 1995). According to statistics, there were 1258 centralized wastewater treatment plants and 5.5 million tons of dry solid sludge produced in China in 2010. About 45% of the sludge produced is currently applied in agriculture (Chen *et al.* 2012).

In this study, we used a sewage sludge that complied with Chinese agricultural standards to increase the soil fertility of mudflat soils. The benefits are two-fold. On the one hand, it saves lands for landfill of sewage sludge and reduces carbon (C) emissions through sludge incineration; on the other hand, it provides an efficient way to improve mudflat soil quality. Past research has mainly focused on the application of sewage sludge in farmlands (Sanchez-Monedero *et al.* 2004; Dichtl *et al.* 2007; Smith 2009; Pritchard *et al.* 2010; San Miguel *et al.* 2012), which showed that land application of sewage sludge increased soil OM (Kladivko and Nelson 1979; Bai *et al.* 2012), yield of plants (Qasim *et al.* 2001; Morera *et al.* 2002), and heavy metal accumulation in plants (Bozkurt and Yarilgac 2003). However, the land application of sewage sludge to mudflats has received little attention. The effect and mechanism of sewage sludge amendment (SSA) in mudflats are quite different because farmlands are different from mudflats in soil nutrient, soil structure, background levels of heavy metals and microbial flora, etc. (Singh and Kar 2001; Mallol 2006; Wang and Wall 2010). The goal of this study was to assess the land application of sewage sludge complying with Chinese agricultural standards as a mud flat soil amendment. Specifically, the effects of SSA on soil chemical properties, metal accumulation and growth of perennial ryegrass (*Lolium perenne* L.) were investigated.

MATERIAL AND METHODS

Study area

The experiment was conducted at the experimental farm of Senmao Company Ltd located in Rudong county, Jiangsu Province, China (E 121°23′23″, N 32°20′03″). This site is a newly reclaimed (4-year-old) mudflat located in the north shore of the Yangtze River estuary. The experiment area is flat with an elevation of 3.00 m above sea level. The region is characterized by subtropic humid monsoon climate with distinct seasons. Precipitation is mainly concentrated from June to August.

Experimental materials

The experimental mudflat soil was typic halaquepts subgroups, which belonged to the halaquepts group of aquepts suborder in inceptisols order based on USDA soil taxonomy. The experimental sewage sludge was collected from the Sewage Treatment Plant of Rudong County in September 2011. The properties of sewage sludge are representative of ordinary sewage sludge pro-duced in China. The chemical properties of mudflat soil and sewage sludge are shown in Table 1. The properties

Table 1 Basic properties of mudflat soil and sewage sludge used in this study

Items	Mudflat soil	Sewage sludge	Municipal wastewater treatment plant-Control standards for agricultural use in China (GB/T 24600-2009)
pH	9.02	6.32	
Salinity (‰)	8.51	32.9	
Organic Matter (g kg^{-1})	3.43	376.9	
Total N (*N* g kg^{-1})	0.282	51.2	
Total P (*P* g kg^{-1})	0.507	5.51	
Alkaline N (*N* mg kg^{-1})	17.08	3440	
Available P (*P* mg kg^{-1})	6.99	813	
Total Mn (mg kg^{-1})	153.1	129.5	
Total Cu (mg kg^{-1})	15.9	1121.9	1500
Total Zn (mg kg^{-1})	56.2	2127.3	4000
Total Ni (mg kg^{-1})	30.9	52.8	200
Total Cd (mg kg^{-1})	1.6	3.3	10
Total Cr (mg kg^{-1})	66.4	155.7	1000

N, nitrogen; P, phosphorus; Mn, manganese; Cu, copper; Zn, zinc; Ni, nickel; Cd, cadmium; Cr, chromium.

of sewage sludge complied with the standard of sludge disposal from municipal wastewater treatment plant control standards for agricultural use in China (GB/T 24600–2009) as shown in Table 1.

Experimental design

The experiment was carried out in a randomized complete block design (RCB) with each plot of 4.0 m length and 4.0 m width. There were five treatments, i.e., 0, 30, 75, 150, and 300 t ha^{-1} SSA rates, and each treatment had triplicates. With the help of a rototiller, the sewage sludge was mixed uniformly with soil down to a depth of 20 cm on October 20, 2011. Perennial ryegrass (*Lolium perenne* L.) as a popular high-quality forage and green manure was chosen for the experimental work, and sowed at 35 g per plot on October 25, 2011. Soil and plant samples were collected for analysis 60 d after sowing.

Soil analysis

Soil samples were collected in quadruplicate from control, 30, 75, 150 and 300 t ha^{-1} SSA. The soil samples were air-dried, crushed, passed through two sieves of 1- and 0.150-mm mesh size and then stored separately for further physicochemical analysis. For analysis of OM content in soil samples, 0.3 g air-dried sample was put through the 0.150-mm mesh and measured by the potassium dichromate ($K_2Cr_2O_7$) method, and total soluble salt in soil samples was measured by the gravimetric method as described by the Soil and Agro-Chemistry Analysis (Bao 2000). The pH of samples was measured in suspension of 1:5 (weight/volume) with a pH meter (Model IQ150, Spectrum, USA), and electric conductivity (EC) by conductivity meter (Item 2265FS, Patent, USA). The cation exchange capacity (CEC) of soil was measured using the ammonium acetate (NH_4OAc) method described by Bao (2000). Exchangeable potassium ions (K^+), sodium ions (Na^+), calcium ions (Ca^{2+}) and magnesium ions (Mg^{2+}) in soil were extracted using the repeated leaching procedure described by Bao (2000). Soil total N and total P contents in the samples were determined by the Semi-micro Kjeldahl method and the Digestion Mo-Sb Anti spectrophotometric method, respectively. The alkaline hydrolysis diffusion method and the sodium bicarbonate ($NaHCO_3$) extraction method were used for the quantification of alkaline N and available P in the samples. For analysis of total heavy metals in soil samples, 0.5 g air-dried sample was put through the 0.150-mm mesh and digested in 20 mL of tri acid mixture (HNO_3:H_2SO_4:$HClO_4$ 5:1:1) for 8 h at 80°C, and each sample had duplicates. After complete digestion solution was filtered and the filtrate was analyzed separately for manganese (Mn), copper (Cu) and nickel (Ni) using a Flame Atomic Absorption Spectrometer (FAAS) (Model SOLAAR M6, Thermo Elemental, Thermo Fisher Scientific Inc., USA), and the filtrate of 10-fold dilution rate for cadmium (Cd) and chromium (Cr) using a Graphite Furnace Atomic Absorption Spectrometer (GFAAS). The quantitative limits of the FAAS system for Mn, Cu, zinc (Zn) and Ni were 0.029, 0.041, 0.013 and 0.063 mg L^{-1}, and those of the GFAAS system for Cd and Cr were 0.028 and 0.036 μg L^{-1}, respectively. The filtrate at 0, 30 and 75 SSA rates and filtrate of five-fold dilution rate at 150 and 300 SSA rates were analyzed for Zn using FAAS. Available metals were analyzed by diethylene triamine pentaacetic acid (DTPA) extraction method. 25.0 g air-dried sample through 1-mm mesh size was extracted in 50 ml DTPA solution for 2 h. After the complete extraction solution was filtered and the filtrate was analyzed separately for Cu and Ni using FAAS, the filtrate of five-fold dilution rate was analyzed for Mn using FAAS, the filtrate at control and filtrate of 50-fold dilution rate at SSA rates was analyzed for Zn using FAAS, and the filtrate of 10-fold dilution rate was analyzed for Cd and Cr using GFAAS.

Plant analysis

Forty plants were selected randomly from each treatment for biomass determination and metal accumulation. For biomass determination, plants were separately washed to remove soil particles adhering on them and then separated into roots and shoots, killed at 105°C for 15 minutes, and oven-dried at 80°C till constant weight was achieved. The plant parts were then weighed separately and biomass accumulation was expressed as g $plant^{-1}$. For heavy metal analysis, oven-dried samples were homogenized by grinding in a stainless steel blender then passed through a sieve of 2-mm mesh size. For extraction of metals (Ni, Cu, Cd, Cr, Zn, Mn) from plant samples, 0.5 g oven-dried sample was digested in 10 mL HNO_3:H_2SO_4:$HClO_4$ (5:1:1) until transparent color appeared (Bao 2000). Metal concentrations were determined after filtering the digested samples using FAAS.

Statistical analysis

The data were analyzed based on analysis of variance (ANOVA) for RCB design using SPSS version 13 software. The least significant difference (Duncan) method at the 0.05 level of significance was performed to test the significant difference between the treatments.

RESULTS

Physicochemical properties of mudflat soil

The effect of SSA on OM content of mudflat soil is shown in Fig. 1. The OM content in mudflat soil

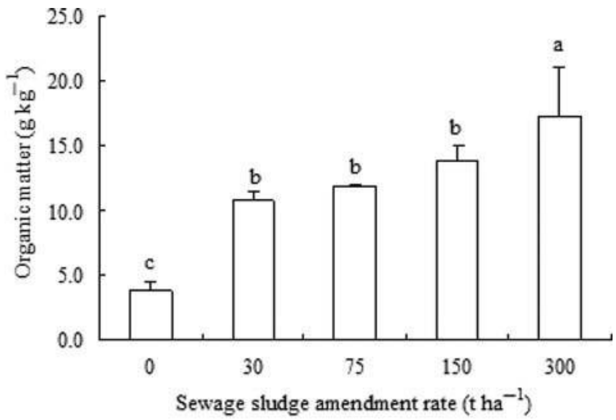

Figure 1 Effect of sewage sludge amendment on organic matter of mudflat soil. Vertical bars indicate standard deviations of the means. Columns with different letters show significant difference between sewage sludge amendment rates at $p < 0.05$ by Duncan's multiple range test.

increased with increasing SSA rates. The OM content in unamended soil was 3.84 g kg^{-1}, compared to 10.80, 11.96, 13.85 and 17.22 g kg^{-1}, which corresponded to 1.8, 2.1, 2.6 and 3.5-fold increase, at 30, 75, 150 and 300 t ha^{-1} SSA rates. However, the increase in OM of mudflat soil was small relative to the increase in SSA rates. The salinity of mudflat soil decreased with increas-ing SSA rates (Fig. 2). The salinity levels at 30, 75, 150 and 300 t ha^{-1} SSA rates were 6.17‰, 3.58‰, 1.83‰and 1.50‰, which corresponded to decreases of 2.6%, 43.4%, 71.0% and 76.3%, compared to 6.33‰ in una-mended soil.

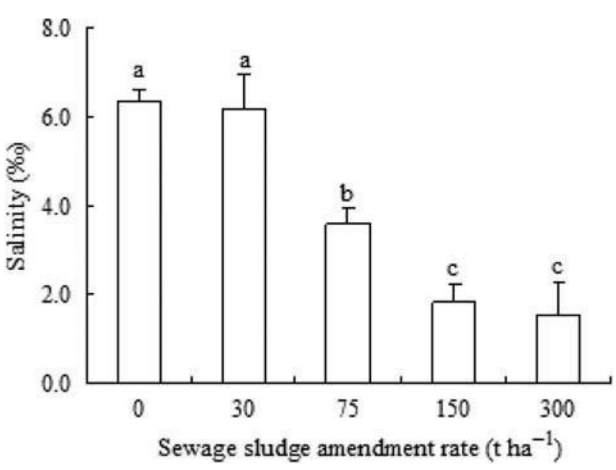

Figure 2 Effect of sewage sludge amendment on salinity of mudflat soil. Vertical bars indicate standard deviations of the means. Columns with different letters show significant difference between sewage sludge amendment rates at $p < 0.05$ by Duncan's multiple range test.

The SSA decreased soil bulk density, and increased soil porosity in comparison to unamended soil (Table 2). Soil bulk density of mudflat soil decreased with increasing SSA rates. Bulk density was lowest at the 300 t ha^{-1} SSA rate, which was a significant difference compared to the other treatments. The increment in soil porosity was 8.32, 7.22, 8.56 and 18.12% at 30, 75, 150 and 300 t ha^{-1} SSA rates, respectively, as compared to unamended soil.

The sewage sludge-amended mudflat soil had lower pH and electric conductivity (EC), and higher cation exchange capacity (CEC), contents of nitrogen (N), phosphorus (P) and exchangeable K^+, Na^+, Ca^{2+} and Mg^{2+} in comparison to unamended soil (Table 2). The pH of mudflat soil decreased with increasing SSA rates. A maximum decrease of 10.2% in pH of mudflat soil was observed at 300 t ha^{-1} rate. The pH of unamended soil was 8.99, compared to 8.07 at the 300 t ha^{-1} SSA rate. EC of mudflat soil decreased with increasing SSA rate, while CEC increased due to SSA amendments. The increments in CEC were 3.52, 4.09, 22.50 and 79.43%at the 30, 75, 150 and 300 t ha^{-1} SSA rates, respectively, as compared to unamended soil. The nutrient contents of mudflat soil increased with increasing SSA rates. The total N, total P, alkaline N and available P in unamended soil were 0.157 g kg^{-1}, 0.607 g kg^{-1}, 24.56 mg kg^{-1} and 8.78 mg kg^{-1}, respectively, compared to 1.212 g kg^{-1}, 0.769 g kg^{-1}, 96.91 mg kg^{-1} and 49.47 mg kg^{-1} at the 300 t ha^{-1} rate. Maximum increases of 672.0, 26.7, 294.6, and 463.4% in total N, total P, alkaline N and available P of mudflat soil were observed at the 300 t ha^{-1} rate. The exchangeable K^+, Na^+, Ca^{2+} and Mg^{2+} concen-trations increased in mudflat soil with SSA.

The SSA increased total Zn and Cu concentrations at the 150 and 300 t ha^{-1} rates ($p < 0.05$), whereas there were either no changes ($p > 0.05$) or decreases ($p < 0.05$) in total Mn, Cr, Ni and Cd concentrations in the sludge-amended soils compared to unamended soil (Table 3). The available metal concentrations for Mn, Cu, Zn, Ni, Cd and Cr increased with SSA ($p < 0.05$). There were sharp increases in soil available Zn and Cu with increasing SSA rates, whereas the available Mn, Ni, Cd and Cr showed only minor increases with increasing SSA rates.

Biomass of perennial ryegrass

The effect of SSA on fresh and dry weight of perennial ryegrass is shown in Fig. 3. Plant biomass was significantly higher in sewage sludge-amended soils than unamended soil ($p < 0.05$). The fresh and dry weights of roots and aboveground parts of perennial ryegrass increased with increasing SSA rates. The fresh weights of aboveground and roots of perennial ryegrass in unamended soil were 0.278 and 0.116 g plant^{-1},

Table 2 Selected chemical properties of mudflat soil at different sewage sludge amendment rates (mean ± 1 standard error)

Parameters	Sewage sludge amendment (t ha^{-1})				
	0	30	75	150	300
Bulk density (g cm^{-3})	1.425 ± 0.034 a	1.373 ± 0.070 a	1.358 ± 0.068 a	1.336 ± 0.062 a	1.198 ± 0.041 b
Porosity (%)	44.54 ± 1.22 c	48.24 ± 2.38 b	47.76 ± 2.28 bc	48.35 ± 2.06 b	52.61 ± 1.35 a
pH (5:1)	8.99 ± 0.26 a	8.93 ± 0.10 ab	8.63 ± 0.08 b	8.25 ± 0.20 c	8.07 ± 0.21 c
EC (ms cm^{-1})	2.29 ± 0.53 a	2.26 ± 0.35 a	1.48 ± 0.29 b	0.71 ± 0.23 c	0.65 ± 0.35 c
CEC (cmol kg^{-1})	5.25 ± 0.16 c	5.43 ± 0.09 bc	5.46 ± 0.38 bc	6.43 ± 1.15 b	9.42 ± 0.48 a
Total N (N g kg^{-1})	0.157 ± 0.040 d	0.485 ± 0.077 cd	0.758 ± 0.221 bc	0.935 ± 0.351 ab	1.212 ± 0.295 a
Total P (P g kg^{-1})	0.607 ± 0.021 c	0.625 ± 0.039 c	0.661 ± 0.019 bc	0.816 ± 0.132 a	0.769 ± 0.046 ab
Alkaline N (N mg kg^{-1})	24.56 ± 5.98 b	37.66 ± 9.33 b	52.23 ± 29.18 b	63.57 ± 29.88 ab	96.91 ± 26.20 a
Available P (P mg kg^{-1})	8.78 ± 1.61 c	15.46 ± 1.08 bc	23.62 ± 4.21 b	42.15 ± 8.80 a	49.47 ± 8.42 a
Ex. K (mg kg^{-1})	255.6 ± 25.5 b	225.0 ± 26.0 b	263.9 ± 6.4 b	266.7 ± 23.6 ab	308.3 ± 29.2 a
Ex. Na (mg kg^{-1})	729.2 ± 67.2 c	927.1 ± 147.3 b	1000.0 ± 70.5 b	1048.6 ± 67.2 ab	1197.9 ± 67.8 a
Ex. Ca (mg kg^{-1})	4927 ± 224 b	5537 ± 225 ab	5822 ± 1141 ab	5755 ± 606 ab	6124 ± 461 a
Ex. Mg (mg kg^{-1})	290.3 ± 15.7 d	311.3 ± 24.2 cd	337.5 ± 7.5 bc	362.4 ± 19.1 b	406.5 ± 25.3 a

Different letters in each row meant significant difference at $p < 0.05$ by Duncan's multiple range test. EC, electric conductivity; CEC, cation exchange capacity; N, nitrogen; P, phosphorus; Ex., exchangeable; K, potassium; Na, sodium; Ca, calcium; Mg, magnesium.

respectively, compared to 1.824 and 0.266 g $plant^{-1}$ at the 300 t ha^{-1} SSA rate. Increases of 98.0, 146.6, 291.4 and 429.2% in fresh weight and 92.5, 132.4, 258.6 and 418.9% in dry weight were observed at the 30, 75, 150 and 300 t ha^{-1} SSA rates, respectively.

Metal uptake

The effect of SSA on metal concentrations in roots and aboveground parts of perennial ryegrass is shown in Fig. 4. The metal concentrations in roots of perennial ryegrass were higher than those of aboveground parts in all SSA rates. Ni, Cu, Cd, Cr, Zn and Mn concentrations in perennial ryegrass grown in sewage sludge-amended soils were significantly higher as compared to those in unamended soil. Cd, Cr and Zn concentrations in aboveground parts and roots of perennial ryegrass showed a monotonic increase with increasing SSA. Ni and Cu concentrations in aboveground parts of perennial ryegrass increased with increasing SSA rates, while Mn concentration in aboveground parts of perennial rye-grass did not change significantly. Ni, Cu and Mn concentrations in root parts of perennial ryegrass increased with increasing SSA rates initially, peaked at the 150 t ha^{-1} SSA rate and then decreased at the 300 t ha^{-1} SSA rate. The metal content in perennial ryegrass from SSA was highest for Zn, followed by Cr, Mn, Cu, Cd and Ni. The effects of sewage sludge application on metal accumulation in perennial ryegrass are shown in Fig. 5. All metal accumulation increased with increasing SSA rates. The metal accumulation for Ni, Cu, Cd, Cr, Zn and Mn in perennial ryegrass was 0.202, 0.492, 0.266, 0.44, 3.94 and 2.95 μg $plant^{-1}$, compared to 1.544, 5.398, 4.059, 24.37, 118.47 and 13.99 μg $plant^{-1}$, which corresponded to 6.6, 10.0, 14.3, 54.0, 29.1 and a 3.8-fold increase, at the 300 t ha^{-1} SSA rate. The correlation analysis showed that Ni, Cu, Cd, Cr, Zn and Mn accumulations in perennial ryegrass grown in sewage sludge-amended soils were positively correlated with SSA rates ($p < 0.05$). The best-fit Linear equations were $y = 0.0044x + 0.2386$ ($R^2 = 1.00$, $p < 0.01$), $y = 0.0163x + 0.65$ ($R^2 = 0.98$, $p < 0.01$), $y = 0.0125x + 0.190$ ($R^2 = 0.99$, $p < 0.01$), $y = 0.0809x - 0.3928$ ($R^2 = 0.99$, $p < 0.01$), $y = 0.3831x - 0.3564$ ($R^2 = 0.99$, $p < 0.01$) and $y = 0.0358x + 4.8922$ ($R^2 = 0.82$, $p < 0.05$), for Ni, Cu, Cd, Cr, Zn and Mn, respectively.

DISCUSSION

Sludge amendment to the mudflat soil improved its chemical properties. The reclaimed mudflat without soil amendment is not suitable for agriculture due to its high salinity and low OM contents (Jones *et al.* 1993).

Table 3 Total and available heavy metal concentrations (mg kg^{-1}) in mudflat soil at different sewage sludge amendment rates (mean ± 1 standard error)

	Sewage sludge amendment (t ha^{-1})				
Parameters	0	30	75	150	300
Total Mn	153.9 ± 20.8 a	105.0 ± 16.6 b	114.7 ± 11.1 ab	150.5 ± 27.6 a	105.3 ± 15.6 b
Total Cu	15.0 ± 1.1 b	15.1 ± 1.1 b	18.5 ± 1.4 ab	20.0 ± 1.7 a	22.2 ± 3.8 a
Total Zn	55.4 ± 2.8 b	78.0 ± 17.3 b	106.2 ± 15.4 b	183.7 ± 27.7 a	202.5 ± 68.8 a
Total Ni	28.1 ± 3.4 a	19.2 ± 4.4 bc	22.3 ± 1.8 abc	26.2 ± 5.3 ab	17.3 ± 3.6 c
Total Cd	1.75 ± 0.10 a	1.41 ± 0.15 b	1.47 ± 0.06 ab	1.54 ± 0.30 ab	1.42 ± 0.07 b
Total Cr	63.1 ± 2.4 a	49.4 ± 5.1 b	54.5 ± 1.7 ab	62.0 ± 8.2 a	52.0 ± 3.7 b
Available Mn	37.88 ± 1.20 ab	36.54 ± 2.29 b	36.52 ± 2.25 b	38.93 ± 1.79 ab	41.17 ± 1.96 a
Available Cu	0.49 ± 0.18 c	0.51 ± 0.09 c	3.20 ± 0.14 b	4.62 ± 1.32 b	7.18 ± 0.67 a
Available Zn	0.55 ± 0.10 b	16.90 ± 7.04 b	27.71 ± 7.95 b	69.29 ± 21.04 a	92.92 ± 27.02 a
Available Ni	0.413 ± 0.088 b	0.363 ± 0.020 b	0.434 ± 0.042 b	0.638 ± 0.092 a	0.734 ± 0.151 a
Available Cd	0.050 ± 0.013 bc	0.045 ± 0.005 c	0.054 ± 0.002 bc	0.065 ± 0.008 ab	0.075 ± 0.017 a
Available Cr	0.328 ± 0.029 ab	0.305 ± 0.028 b	0.332 ± 0.025 ab	0.336 ± 0.024 ab	0.367 ± 0.021 a

Different letters in each row meant significant difference at $p < 0.05$ by Duncan's multiple range test. Mn, manganese; Cu, copper; Zn, zinc; Ni, nickel; Cd, cadmium; Cr, chromium.

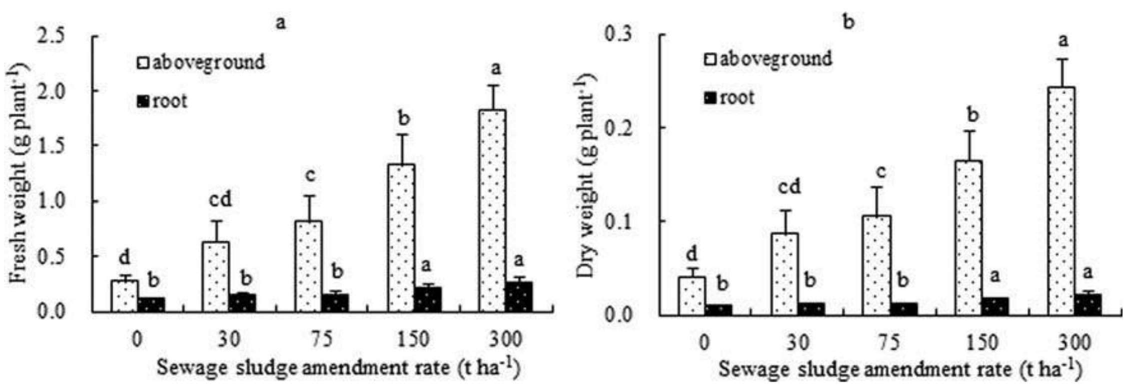

Figure 3 Effect of sewage sludge amendment on fresh (a) and dry weight (b) of perennial ryegrass (*Lolium perenne* L.). Vertical bars indicate standard deviations of the means. Columns with different letters show significant difference between sewage sludge amendment rates at $p < 0.05$ by Duncan's multiple range test.

Sewage sludge, on the other hand, is rich in OM and inorganic nutrients and may be a good nutrient source for crops. Previous studies have shown that the SSA can increase soil OM content significantly (Wang *et al.* 2008; Castro *et al.* 2009), reclaim mine tailing-contaminated soils (Domene *et al.* 2009), amend calcareous soils (Hemmat *et al.* 2010), modify soil physicochemical and biological properties (Singh and Agrawal 2008) and improve physicochemical properties of saline soil, especially C and N contents (Lakhdar *et al.* 2010b). This study demonstrated that the SSA increased OM content of mudflat soil, and soil OM increased with increasing SSA rate. However, the increment of OM of mudflat soil in response to SSA was small compared to the increment of SSA rates. The fraction of the added OM that persists after 1 year is termed the humification coefficient (Janssen 1984). Humification coefficients during three months of this study decreased with increasing SSA rate, which were 74.6, 46.0 and 30.8% at 75, 150 and 300 t ha^{-1} SSA rates, respectively. This result suggests that the more OM added, the more C lost in amended mudflat soils. The SSA reduced the pH of mudflat soil in this study, probably due to acidity of the sewage sludge. Previous studies have also found lowering of pH with SSA (Moreno *et al.* 1997; Singh and Agrawal 2007; Singh et al. 2010a). In addition, release of humic acid from biodegradation of OM-rich sewage sludge may contribute to lower soil pH at SSA (Moreno *et al.* 1997). Soil N and P contents and exchangeable K^+, Na^+, Ca^{2+} and Mg^{2+} increased in the mudflat soil amended with sewage sludge due to higher levels of these nutrients in the sewage sludge. Soil fertility improvement by SSA has been reported widely (Moreno *et al.* 1997; Singh and Agrawal 2007; Wang

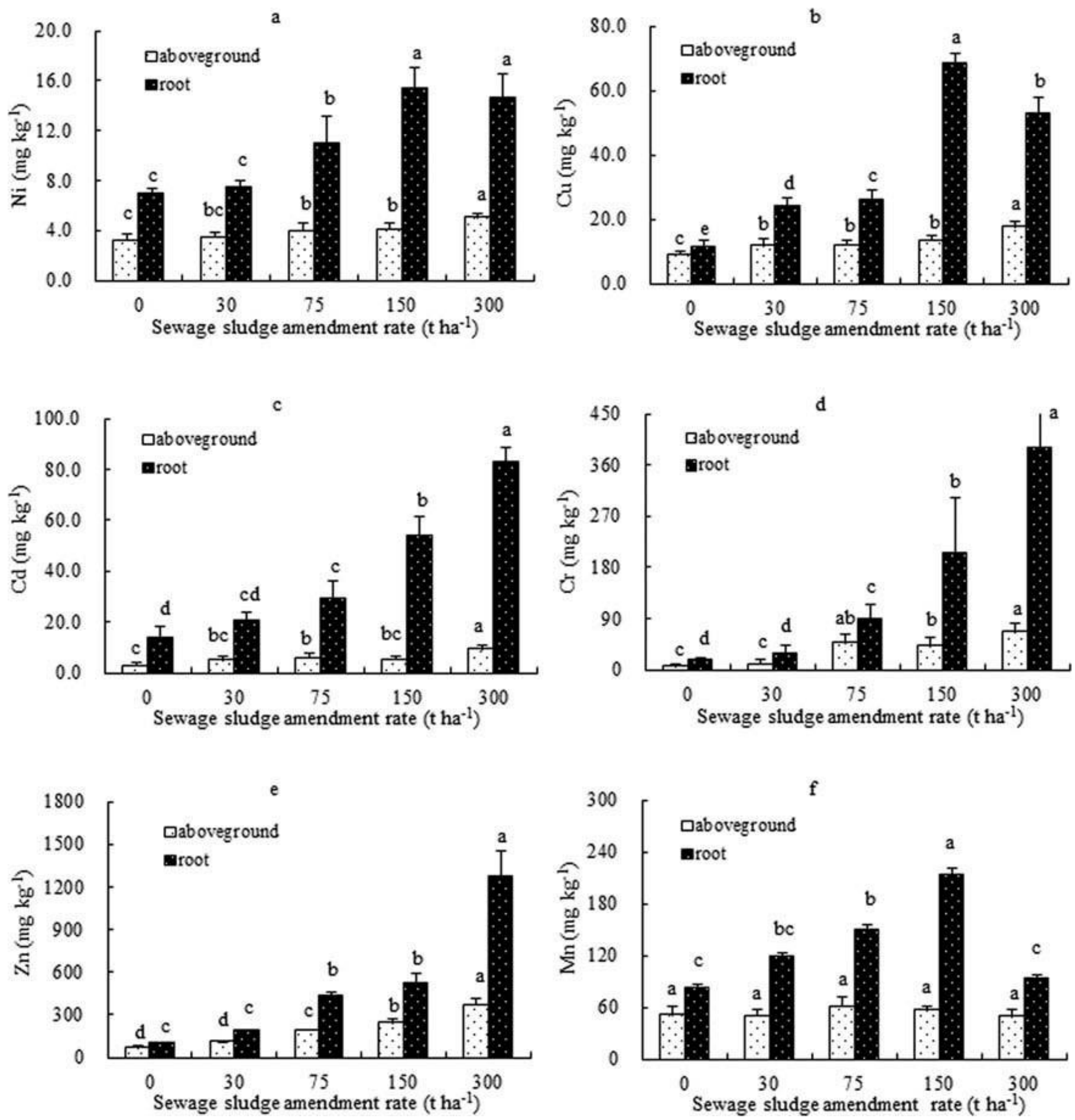

Figure 4 Effect of sewage sludge amendment on heavy metal concentration for (a) nickel (Ni), (b) copper (Cu), (c) cadmium (Cd), (d) chromium (Cr), (e) zinc (Zn) and (f) manganese (Mn) of perennial ryegrass (*Lolium perenne* L.). Vertical bars indicate standard deviations of the means. Columns with different letters show significant difference between sewage sludge amendment rates at $p < 0.05$ by Duncan's multiple range test.

et al. 2008; Singh *et al.* 2010a). The SSA increased CEC of mudflat soil, probably due to increasing soil OM content (Chaney and Swift 1984; Goldberg *et al.* 1988). The SSA also improved soil physical properties by increasing porosity and decreasing bulk density. As a result, the salinity of the mudflat soil dropped. The high salinity in mudflat soils is caused by capillary rise that brings salts to the soil surface (Jorenush and Sepaskhah 2003). Breaking capillary rise is an effective way to decrease soil salinity. in particular, an increase in non-capillary porosity might facilitate the downward salt leaching by rainfall while blocking upward salt movement through capillary rise (Xie *et al.* 1993). Soil aggregates can increase non-capillary porosity. Previous studies found SSA increased OM content and thus increased soil aggregates in a clay loam brown forest soil (Hamuda and Ligetvari 2011). The study on sandy loam, entisol and alfisol soils also showed that SSA increased the amount of macro-aggregates (Sandoval-Estrada *et al.* 2010; G r o s b e l l e t *et al.* 2011). Therefore, salinity reduction by SSA might be attributed to the fact that OM enrichment by SSA reduced soil bulk density and increased soil porosity. Correlation

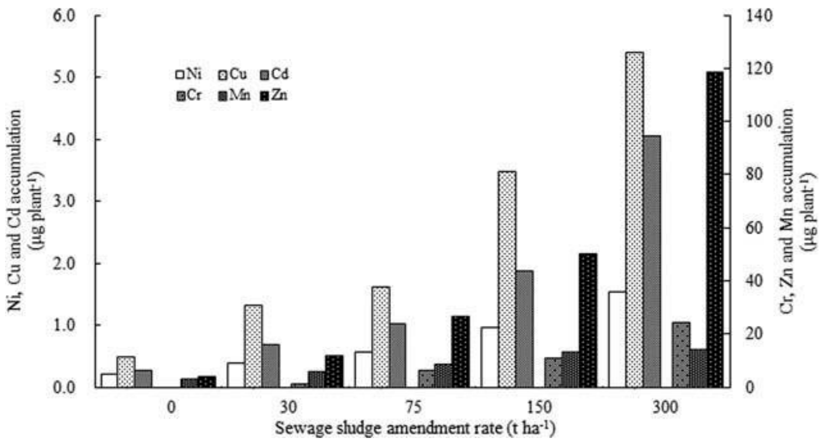

Figure 5 Effect of sewage sludge amendment on heavy metal accumulation for nickel (Ni), copper (Cu), cadmium (Cd), chromium (Cr), zinc (Zn) and manganese (Mn) in perennial ryegrass (*Lolium perenne* L.).

analysis showed that salinity of mudflat soil correlated negatively with OM content. The non-linear fit equation $Y = 12.479e^{-0.1156X}$ (R^2 = 0.7291) was proposed, which was consistent with the results of previous studies (Xie *et al.* 1993).

The SSA enhanced the growth of perennial ryegrass plants in mudflat soil. Uzun and Bilgili's study (2011) also con- firmed that the SSA increased the number of emergences, number of seedlings, plant height and clipping yield of perennial ryegrass. The SSA also enhanced shoot and root biomass of sorghum-sudan grass (*Sorghum vulgaris* var. Sudanese hitche) 2- to 3-fold (Sivapatham *et al.* 2012). Others found that SSA increased the yields of maize (*Zea mays* L.) (Jamali *et al.* 2008), durum wheat (*Triticum durum* Desf.) (Tamrabet *et al.* 2009), spinach (*Spinacea oleracea* L.)(Ngole 2010), cowpea (*Vigna unguiculata* L.) plants (Santos *et al.* 2011), kenaf cultivar (*Hibiscus cannabinus* L.) (Andres *et al.* 2010) and bouteloua species (*Bouteloua gracilis* Lag. and *Bouteloua scorpioides* Lag.) (Lara-Villa *et al.* 2011). Singh and Agrawal (2010b) found that the SSA increased height, leaf number, leaf area, total biomass and yield of rice (*Oryza sativa* L.). The present study showed that SSA increased the biomass of roots and aboveground parts of perennial ryegrass grown in mudflat soil. The growth enhancement might be the consequence of improved physi-cochemical properties of mudflat soil by SSA including increased OM content and decreased bulk density and pH of mudflat soil (Bai *et al.* 2012). In addition, mudflat soil was enriched with high-quality OM, N, P and other nutrients from SSA (Zhou *et al.* 1999; Qiao and Luo 2001), which provided sufficient nutrients for perennial ryegrass growth.

The SSA increased Ni, Cu, Cd, Cr, Zn and Mn con-centrations in perennial ryegrass grown in the mudflat soil. Previous studies found that the sewage sludge increased Ni and lead (Pb) uptake in broccoli (*Brassica oleracea* L.) plants (Antonious 2009), Zn and Cu contents in wheat (*Triticum aestivum* L.) grains (Li *et al.* 2012), Cu and Mo contents in shoot of soybean (*Glycine max* L.) (Sridhar *et al.* 2011), Zn and Cu in pepper (*Capsicum annuum* L.) fruit (Azcona *et al.* 2011). In our study, metal concentrations in roots were higher than in aboveground parts. The metal accumulation in perennial ryegrass correlated positively with SSA rates. Erdogan's (2011) study also confirmed that sewage sludge increased metal accumulation in ornamental plants and accumulation in roots was higher than in aboveground parts. The metal accumulation in perennial ryegrass grown in mudflat soil was determined by avail-able metal concentrations of mudflat soil, which were increased by SSA. This study showed that the accumula-tion of Ni, Cu, Cd, Cr and Zn in perennial ryegrass showed positive correlations with available metal con-centrations in mudflat soil ($p < 0.05$), except for Mn (Fig. 6). The best-fit Linear equations were $y = 3.1638x - 0.9017$ ($R^2 = 0.90$, $p < 0.05$), $y = 0.6642x + 0.3369$ ($R^2 = 0.92$, $p < 0.01$), $y = 116.85x - 5.1954$ ($R^2 = 0.89$, $p < 0.05$), $y = 403.6x - 126.09$ ($R^2 = 0.84$, $p < 0.05$) and $y = 1.1332x - 4.8311$ ($R^2 = 0.89$, $p < 0.05$) for Ni, Cu, Cd, Cr and Zn, respectively. Mn accumulation in per-ennial ryegrass did not show positive correlation with available Mn concentrations in mudflat soil, which was due to the small variance of soil available Mn among treatments.

In this study, the perennial ryegrass growth was not inhibited by heavy metal accumulation even at high doses of SSA. In contrast, some studies showed that biomass increased and then decreased with SSA rates for fescue (*Festuca ovina* L.) and perennial ryegrass (Hua *et al.* 2007), green gram (*Phaseolus mungo* L.) (Chandra *et al.* 2008) and wheat (Lakhdar *et al.* 2010a). The study in pepper plants showed that dry weight of leaf, shoot and root, fruit yield and number

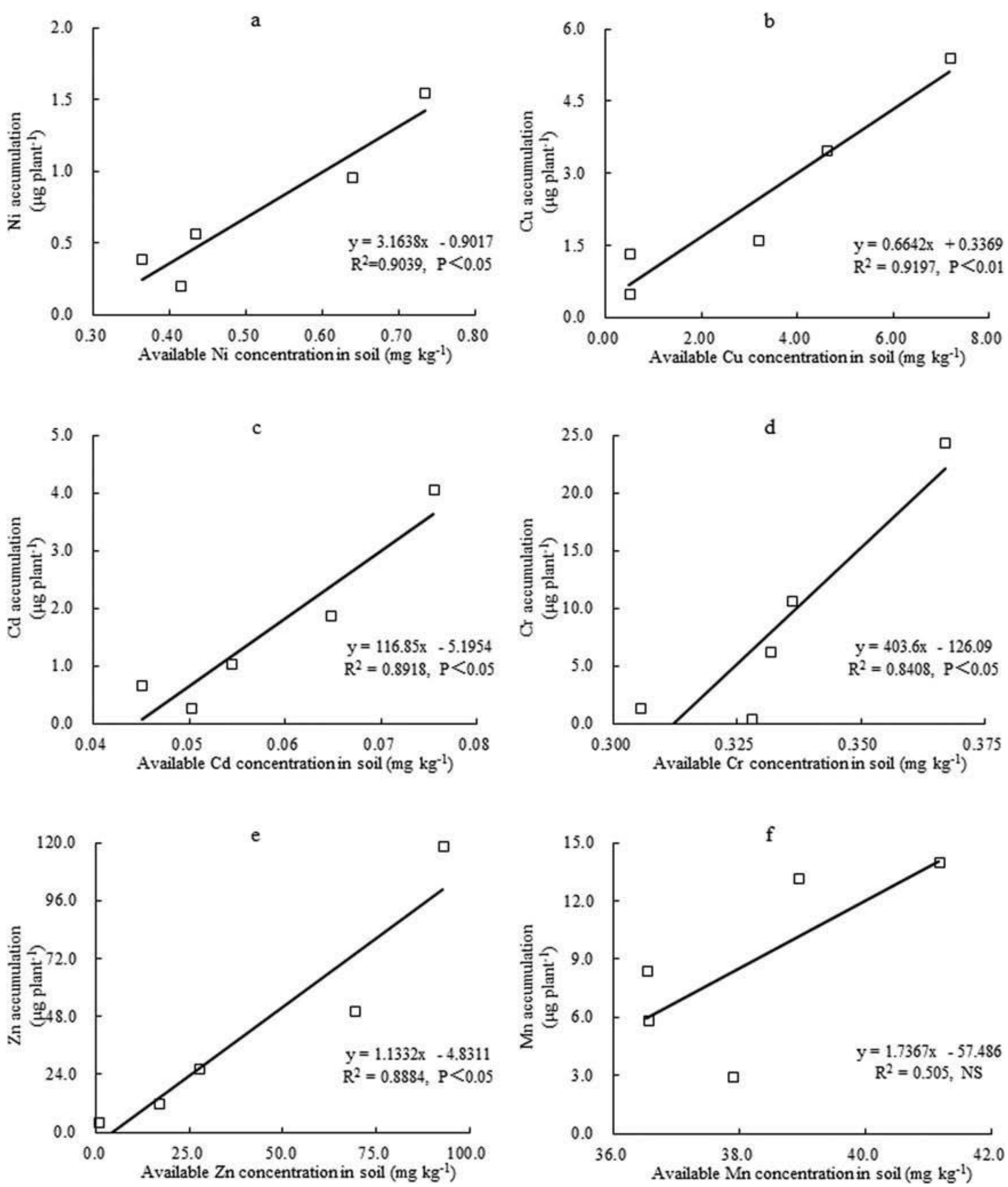

Figure 6 Relationships between available metal concentrations in mudflat soil and metal accumulation for (a) nickel (Ni), (b) copper (Cu), (c) cadmium (Cd), (d) chromium (Cr), (e) zinc (Zn) and (f) manganese (Mn) in perennial ryegrass (*Lolium perenne* L.).

of fruits per plant increased at first and then decreased with increasing SSA rates (Pascual *et al.* 2010). In those studies, the negative effect of SSA on plant yield was caused by heavy metal accumulation in the plants (Singh and Agrawal 2008; Wang *et al.* 2008; Pritchard *et al.* 2010). The lack of inhibition effect of metal accumulation on perennial ryegrass growth in this study might be due to the relatively low metal accumulation in the plant, which was attributed to the type of sludge used. Sewage sludge used in this study complied with Chinese agriculture standards and thus was relatively low in metal contents compared to those previous studies (Singh and Agrawal 2008; Wang *et al.* 2008; Pritchard *et al.* 2010). However, one needs to be cautious about sewage sludge amendment of mudflats with respect to potential metal accumulation in plants. The potential heavy metal transfer through the food chain needs further investigation.

CONCLUSIONS

The application of sewage sludge improved the physicochemical properties of mudflat soil by decreasing soil alkalinity, EC and bulk density, and increasing soil porosity, CEC, N and P contents and exchangeable K^+, Na^+, Ca^{2+} and Mg^{2+}. Fresh and dry weights of perennial ryegrass significantly increased with increasing SSA rates. The SSA increased metal concentrations of aboveground and root parts of perennial ryegrass. The metal content in perennial ryegrass was highest for Zn, followed by Cr, Mn, Cu, Cd and Ni, and the concentrations of the metals in roots were significantly higher than in the aboveground parts of perennial ryegrass. The metal accumulation in perennial ryegrass correlated positively with sludge application rates and available metal concentrations in mudflat soil.

The results of the present study suggest that mudflats amended by sewage sludge might be a promising method for both mudflat reclamation and disposal of sewage sludge. However, heavy metal accumulation in plants may cause food chain contamination concerns. Further studies on crop plants are warranted to assess human health risks.

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